I HEREBY CERTIFY THAT THIS CORRESPONDENCE IS BEING DEPOSITED WITH THE UNITED STATES POSTAL SERVICE AS FIRST CLASS MAIL IN AN ENVELOPE ADDRESSED TO: ASSISTANT COMMISSIONER FOR PATENTS, WASHINGTON, D.C. 20231, ON THE DATE INDICATED BELOW.

BY: Lotte C. Kristman DATE: December 10, 2001

PATENT
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re:	Patent Application of Ross *et al.*	: Group Art Unit: 1642
Appln. No:	09/961,086	: Examiner: Not Yet Assigned
Filed:	September 21, 2001	: Attorney Docket No. **10460-7U1**
For:	BREAST CANCER RESISTANCE PROTEIN (BCRP) AND THE DNA WHICH ENCODES IT	: (053836-5001-01)

PRELIMINARY AMENDMENT

The Applicants hereby respectfully request that the present Amendment be entered prior to issuance of the present application.

In the Specification

Please delete the section of the specification entitled "Brief Description of the Drawings" and substitute in place thereof the amended section submitted herewith.

REMARKS

Substitute drawing sheets were required in the Notice to File Corrected Application Papers dated September 21, 2001. Formal Drawings are being filed concurrently with this Amendment, along with a marked up copy of the amended "Brief Description of the Drawings" section and a clean copy of the amended section to be substituted for the requested deletion.

The foregoing amendment to the specification revises the description of each of Figures 1C, 2C, 3, 4A, 4D, and 7, so that the legends accurately reflect the content of the

drawings. In order to formalize some of the Figures, text was removed from the drawings and inserted into the legends. Thus, these amendments add no new matter to the specification.

Respectfully submitted,

DOUGLAS D. ROSS

December 19, 2001
(Date)

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KD/JAI
Enclosures: 25 Formal Drawing Sheets
1 Transmittal of Formal Drawings
1 Marked Up Copy of "Brief Description of the Drawings" Section (2 pages)
1 Clean Copy of "Brief Description of the Drawings" Section (2 pages)

MARKED UP COPY

Brief Description of the Drawings

Figure 1A is an autoradiograph of the RNA fingerprinting of MCF-7 cells.

Figure 1B is an autoradiograph of a Northern blot hybridization of mRNA from MCF-7/W (W), MCF-7/ AdrVp (AdrVp), and MCF-7/AdrVpPR (AdrVpPR) cells.

Figure 1C is an autoradiograph of a genomic Southern blot hybridization of DNA from MCF-7/AdrVp (AdrVp), MCF-7/W (W) and MCF-7/AdrVpPR (AdrVpPR) cells.

Figure 2A is the deduced amino acid sequence of BCRP with motifs.

Figure 2B shows the relative similarity of BCRP to selected members of the ABC transporter superfamily.

Figure 2C, comprising Figures 2C-1, 2C-2, and 2C-3, is the cDNA sequence which encodes the BCRP.

Figure 2D is a graph of a phylogram showing the evolution of the amino acid sequence of BCRP in relation to certain other members of the ABC family of transport proteins.

Figure 3 shows an autoradiograph of a multiple tissue Northern blot. Key to the lane numbering is as follows: heart (1), brain (2), placenta (3), lung (4), liver (5), skeletal muscle (6), kidney (7), pancreas (8), spleen (9), thymus (10), prostate (11), testis (12), ovary (13), small intestine (14), colon (15), peripheral blood leukocytes (16).

Figure 4A is an autoradiograph of a Northern blot of subclones of BCRP transfectants[.] demonstrating expression of BCRP mRNA in subclones of MCF-7/W cells stably transfected with the expression vector pcDNA3-BCRP.

Figure 4B is a graph of Daunorubicin (DNR) accumulation and retention in the pcDNA3 vector control cells and BCRP-transfected clones 6 and 8.

Figure 4C shows the relative resistance factors-MCF-7, vector control, clones 19, 6, and 8.

Figure 4D, comprising Figures 4D-1 through 4D-6, are graphs showing the effect of various chemotherapeutic drugs' concentrations on BCRP-transfected MCF-7 clone 8 cell survival.

Figure 4E shows a graph of the effects of ATP deletion of retention of rhodamine 123 by transfectant MCF-7/pcDNA3 (empty vector control) or MCF-7/BCRP clone 8 cells.

Figure 5 is a table showing the effect of various chemotherapeutic drugs on BCRP-transfected MCF-7 cells.

Figure 6 is an autoradiograph showing the expression of Human ω gene in MCF-7 cells detected by the Reverse Transcription-Polymerase chain reaction (RT-PCR).

Figure 7 is an autoradiograph showing the expression of BCRP in samples of blast cells from patients with acute myelogenous leukemia (AML). Detection of the expression of BCRP mRNA transcripts in MCF-7/W cells or in blast cells from 14 patients with AML is shown. Total cellular RNA was isolated as described previously (10), then 1 μg of RNA was added to a reverse-translation reaction mixture containing AMV reverse transcriptase, and oligonucleotide primers specific for beta-actin (10) or BCRP (See Example 11), as described previously (10). Following reverse-transcription, PCR was performed as described in Example 11, then an aliquot of the PCR reaction mix was subjected to agarose gel electrophoresis. For BCRP, the agarose gel was transferred to nitrocellulose membranes then Southern hybridization was done using ^{32}P-labeled "clone-8 PCR product" as probe for BCRP. A radioautograph of this southern blot is s shown in this figures. M=DNA size marker. The number under MCF-7 indicates the μl of PCR reaction mixture that was added to the agarose gel lane. The numbers 1 tot 14 indicate an AML patient blast cell sample; 15 μl of PCR reaction mix were added per gel lane for the AML samples. For beta actin, the PCR product on ethidium bromide stained gels was approximately equal for the patient samples and for an equivalent amount of MCF-7/W PCR reaction mixture (data not shown).

Figure 8A, 8B, and 8C are autoradiographs showing the results of Northern blot hybridizations of mRNA from various drug resistant cell lines probed with a BCRP probe.

Figure 9 is an autoradiograph of a Southern blot hybridization from various MCF-7 cell lines.

Figure 10 is a graph showing the results of administration of FTC to BCRP transfected cells.

CLEAN COPY

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